20 μ g type IV collagen was lysed in 1 ml phosphate buffered solution (PBS), and this lysate solution was used at 50 μ 1/well, and after coating a 96-well microplate (Falcon; Becton Dickinson Labware) at 4° C for overnight, was washed three times with PBS containing 0.05% Tween 20 and 0.1% BSA, and then blocked with PBS containing 0.2% BSA at 250 μ 1/well at 4° C overnight.

The serum obtained from the blood mentioned above was then diluted to 400 to 20000 times, and the diluted serum was added to the aforementioned 96-well microplate at 50 μ 1/well, and allowed to react at 4° C overnight. After the reaction, the 96-well microplate was washed three times with PBS containing 0.05% Tween 20, added 50 μ 1 of horseradish peroxidase (Sigma Chemical Co.)-conjugated goat anti-mouse IgG1, IgG2a, or IgG2b diluted to 200 times, and was then incubated at 4° C for 2 hours. After incubation, it was washed again three times with PBS containing 0.05% Tween 20, and developed enzyme reaction at room temperature for 30 minutes with 0.1 ml of True Blue Peroxidase Substrate (Kirkegaard & Perry Labs). The OD 450 was then read by using a Microplate Reader (Biolumin960; Molecular Dynamics Japan, Inc.). The results are shown in Fig. 3.